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Cytological Studies of Genus *Boehmeria* Jacq. (Urticaeae) in Nepal

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A cytological study for the species of *Boehmeria* Jacq. (Urticaceae) of Nepal is carried out for the first time. 2n = 28 chromosomes are determined for all the individuals studied. Hence *Boehmeria* species in Nepal Himalaya appear to be diploid and considered to be reproduced sexually in reference to the studies of *Boehmeria* species in Japan where polyploidy and apomixis are common in the species of *Boehmeria*.

Key words: Agamospermy, apomixis, *Boehmeria*, chromosome, polyploidy.

Boehmeria (Urticaceae) is known for the troublesome variability and is one of the most difficult genus for the taxonomist throughout the distribution range, i.e. the tropical to temperate regions of Asia, Oceania, America and some islands in Indian Ocean (Blume 1856, Weddell 1856). Previous cytological studies in Japan (Okabe 1963, Yahara 1983) revealed that the basic chromosome number of the genus is X = 14 and diploid, triploid, tetraploid and pentaploid were known (Table 1), of which diploid plants propagated sexually while polyploids showed agamospermy. In Japan sexual diploids have limited distribution and mostly inhabit natural undisturbed places, while agamospermous polyploids are much more common and prefer disturbed habitat. Frequent occurrence of agamospermous plants originated from hybridization between sexual and other agamospermous taxa are considered to contribute the morphological variability and taxonomical complexity in this genus (Yahara 1983, 1990). Cytological data of this genus outside Japan are still limited and only one record of diploid individual (Yahara 1981) has been hitherto known from Thailand, but studies used presence/absence of male flowers and pollen stainability as indicators of sexuality suggested that the trends of frequency and habitat preference of both sexual and agamospermous taxa similar to Japan were also common in the eastern China (Yahara 1990).

Critical cytological studies in the Himalaya have been mostly carried out in the alpine and subalpine taxa viz: *Saxifraga*, *Primula*, *Impatiens*, *Pedicularis*, *Sedum* and *Sinocrassula* (Wakabayashi 1988, 1997, Wakabayashi and Ohba 1988, 1999, Sakya and Joshi 1990, Akiyama and Wakabayashi 1992, Amano 1997, 1999). Cytological study of a few genera of Urticaceae growing in the montane zone viz: *Girardiana*, *Lecanthus*, *Pilea*, and *Urtica* has been carried out (Sharma 1961). According to these studies polyploidy and apomixis were found in some taxa but the proportion of polyploidy in alpine flora was generally low compared with arctic region (Ohba 1988). However, data is still wanting to discuss whether apomixis plays a role in the floristic diversity in the

Table 1. Reported chromosome numbers of *Boehmeria* of Japan

Plant name	2n	Locality	Reference
B. arenicola	42	Shiogama, Shimoda	Okabe (1963)
B. arenicola	56	Akinohama, Izu Oshima island	Yahara (1983)
B. arenicola	56	Naganezaki, Izu Oshima island	Yahara (1983)
B. biloba	28	Akinohama, Izu Oshima island	Yahara (1983)
B. biloba	28	Matushima, Yotukura	Okabe (1963)
B. dura	56	Akinohama, Izu Oshima island	Yahara (1983)
B. dura	56	Okada, Izu Oshima island	Yahara (1983)
B. dura	56	Motomachi, Izu Oshima island	Yahara (1983)
B. egregia	42	Habuminato, Izu Oshima island	Yahara (1983)
B. egregia	42	Katuura	Okabe (1963)
B. egregia	42	Motomachi, Izu Oshima island	Yahara (1983)
B. hatsusimae	42	Wakasugiyama	Okabe (1963)
B. hispidula	42	Akinohama, Izu Oshima island	Yahara (1983)
B. hispidula	42	Sendai	Okabe (1963)
B. hispidula	42	Motomachi, Izu Oshima island	Yahara (1983)
B. hispidula	42	Naganezaki, Izu Oshima island	Yahara (1983)
B. holosericea	28	Echizenkaigan seacost	Yahara (1983)
B. holosericea	28	Gonoura, southern part of Iki island	Yahara (1983)
B. holosericea	28	Ishida, southern part of Iki island	Yahara (1983)
B. holosericea	28	Nishinoura, northern seacost, Itoshima peninsula	Yahara (1983)
B. holosericea	28	Shiogama	Okabe (1963)
B. izushimensis	56	Akinohama, Izu Oshima island	Yahara (1983)
B. izushimensis	56	Habuminato, Izu Oshima island	Yahara (1983)
B. izushimensis	56	Okada, Izu Oshima island	Yahara (1983)
B. kiusiana	42	Hainzuka	Okabe (1963)
B. longispica	42	Echizenkaigan seacost	Yahara (1983)
B. longispica	56	Echizenkaigan seacost	Yahara (1983)
B. longispica	70	Echizenkaigan seacost	Yahara (1983)
B. longispica	42	Gonoura, southern part of Iki island	Yahara (1983)
B. longispica	56	Gonoura, southern part of Iki island	Yahara (1983)
B. longispica	42	Mashoji, northern seacost of Itoshima peninsula	Yahara (1983)
B. longispica	56	Nishinoura, northern seacost, Itosima peninsula	Yahara (1983)
B. longispica	42	Nogita, northern seacost, Itoshima peninsula	Yahara (1983)
B. longispica	42	Noto Fukuura	Yahara (1983)
B. longispica	42	Tokyo	Okabe (1963)
B. maximowiczii	42	Sendai	Okabe (1963)
B. minor	42	Jimmuji	Okabe (1963)
B. nakashimae	42	Nishinoura, northern seacost, Itoshima peninsula	Yahara (1983)
B. nivea	28	Tokyo	Okabe (1963)
B. nivea var. *nipononivea*	28	Sendai, Takaosan	Okabe (1963)
B. pannosa	28	Shikanoshima	Okabe (1963)
B. paraspicata	28	Sendai, Asahikawa	Okabe (1963)
B. platanifolia	56	Noto Fukuura	Yahara (1983)
B. platanifolia	70	Noto Fukuura	Yahara (1983)
B. platanifolia	42	Takaosan	Okabe (1963)
B. praestabilis	42	Shimoda	Okabe (1963)
B. quelpartensis	42	Echizenkaigan seacost	Yahara (1983)
B. quelpartensis	42	Ishida, southern part of Iki island	Yahara (1983)
B. robusta	42	Yugawara	Okabe (1963)
B. spicata	42	Sendai, Wakasugiyama	Okabe (1963)
B. spicata var. *microphylla*	28	Yugawara, Shimoda	Okabe (1963)
B. tenuifolia	42	Shimoda	Okabe (1963)
B. tricuspis	42	Sendai	Okabe (1963)
B. utilis	28	Sendai	Okabe (1963)

Himalayas. Cytological data of the plants on montane zone and subtropical terai, the most disturbed regions by human interference, are hardly known. Eleven species and seven varieties of *Boehmeria* were reported from Nepal (Acharya 2001), most of which were growing in subtropical montane zones. None of them have been studied cytologically.

Present study is the first cytological study of *Boehmeria* in Nepal Himalaya, which is aimed to clarify: 1. The ploidy level and reproduction mode of *Boehmeria* species in Nepal, 2. The relationship of cytology and morphological diversity in each taxa, 3. Whether apomixis plays an important role in the diversification of *Boehmeria* in Nepal as shown in Japan. This study will provide the basic data on the evolution of plants growing in the montane to subtropical zones in the Himalayas which have been less botanized especially in the field of cytology.

Materials and methods

Sixteen individuals of nine taxa of two sections, Phyllostachys and Duretia among five sections proposed by Wang (1981) under genus *Boehmeria,* were studied. Among other sections, sect. Boehmeria and sect. Tiliocnide, including one species in each in Nepal Himalaya, were not found or failed to collect root-tips during our study and sect. Zollingeriana does not include any taxa from Nepal.

Root tips used for the cytological study were collected during the field trip June 25–July 19, 2000 in Nepal. Voucher specimens were also collected together with the root tips, their respective collection localities, elevation and voucher numbers are presented in Table 2. Voucher specimens are deposited in TUS. Root tips were pretreated in 0.05 % colchicine solution for nearly 3 hours immediately after collection at low temperature and then fixed in Newcommer's fluid (Sharma and Sharma 1980). Fixed materials were hydrated through ethyl-alcohol series. Hydrated root tips were soaked in enzyme solution (2 % pectolyase and 4 % cellulase) for 55 minutes at 37 ℃ to macerate the tissue. The macerated root-tips were stained in 2 % aceto-orcein for 12 hours and squashed to spread the cells and

Table 2. Chromosome numbers of *Boehmeria* of Nepal

Name of the plant	2n	Voucher data: collection locality, elevation and voucher number
B. canescens	28	Lalitpur district, Godawari, 1500 m, NA 2028
B. canescens	28	Bhaktapur district, Thimi, 1400 m, NA 2035
B. clidemioides var. *clidemioides*	28	Rasuwa district, near Pairo, 1410 m, NA 2015
B. densiflora var. *penduliflora*	28	Ilam district, Lodhiyamai, 450 m, NA 2001
B. densiflora var. *penduliflora*	28	Ilam district, Lodhiyamai, 450 m, NA 2002
B. hamiltoniana	28	Kathmandu district, Nagarjun, 1450 m, NA 2034
B. macrophylla var. *macrophylla*	28	Ilam district, Soktim, 600 m, NA 2003
B. macrophylla var. *rotundifolia*	28	Rasuwa district, Syaprubesi, 1400 m, NA 2010
B. macrophylla var. *rotundifolia*	28	Lalitpur district, Phulchoki, 2100 m, NA 2022
B. macrophylla var. *rotundifolia*	28	Lalitpur district, Bhardew, 2160 m, NA 2023
B. macrophylla var. *scabrella*	28	Kathmandu district, Nagarjun, 1550 m, NA 2032
B. polystachya	28	Rasuwa district, near Bamboo, 2100 m, NA 2016
B. polystachya	28	Rasuwa district, Lamahotel, 2100 m, NA 2017
B. polystachya	28	Rasuwa district, Dangdugekhola, 2000 m, NA 2018
B. ternifolia	28	Lalitpur district, Godawari, 1700 m, NA 2027
B. ternifolia	28	Lalitpur district, Godawari, 1700 m, NA 2029

observed the chromosome under light microscope Olympus BX 50F.

Results

Chromosome numbers of nine taxa of genus *Boehmeria* from Nepal Himalaya are shown in Table 2. In all individuals studied 2n = 28 chromosome were observed. The size of the chromosome is approximately 1.5 μm (Figs. 1–3).

Discussion

All the individuals examined in the present study have 2n = 28 chromosomes in their somatic cells which are considered to be diploid. Though the reproduction mode of individuals studied could not be examined directly because the collection season was too early for flowering time of *Boehmeria* species, they are considered as sexual because previous studies (Okabe 1963, Yahara

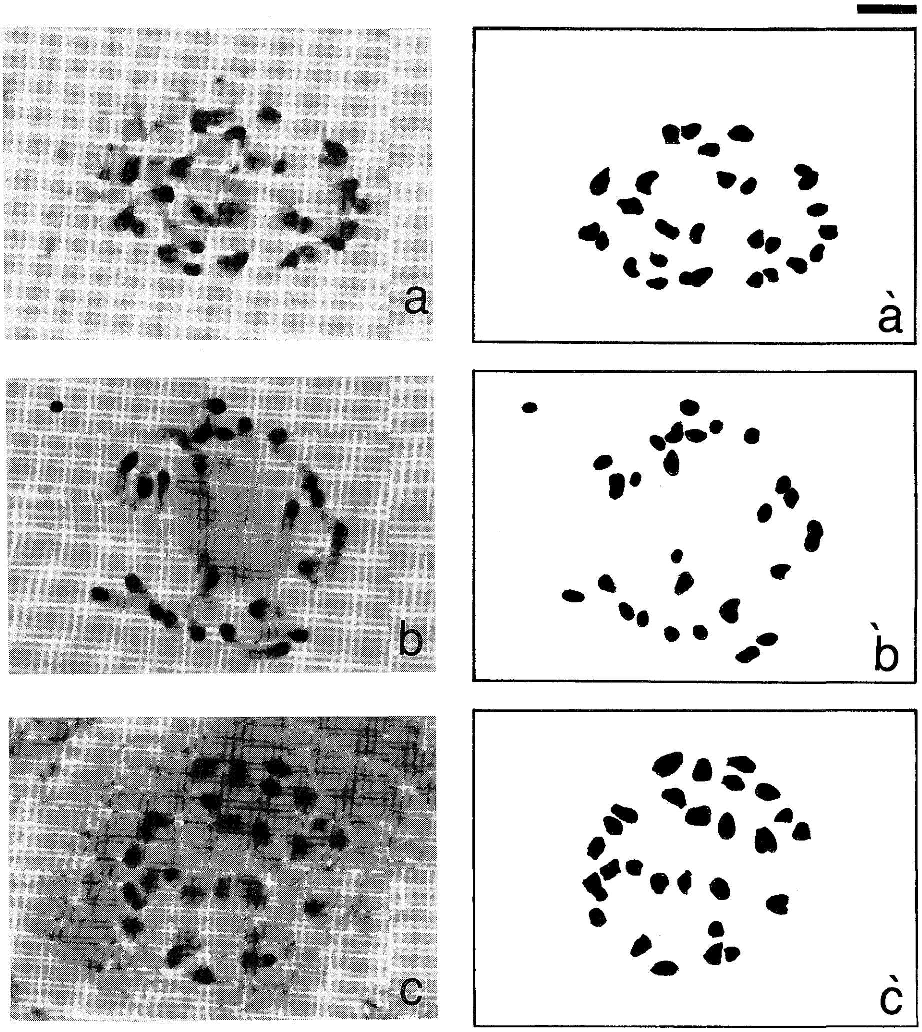

Fig. 1. Chromosomes of *Boehmeria*. a, à. *B. canescens* b, b̀. *B. clidemioides* var. *clidemioides* c, c̀. *B. hamiltoniana*. Scale bar = 4 μm.

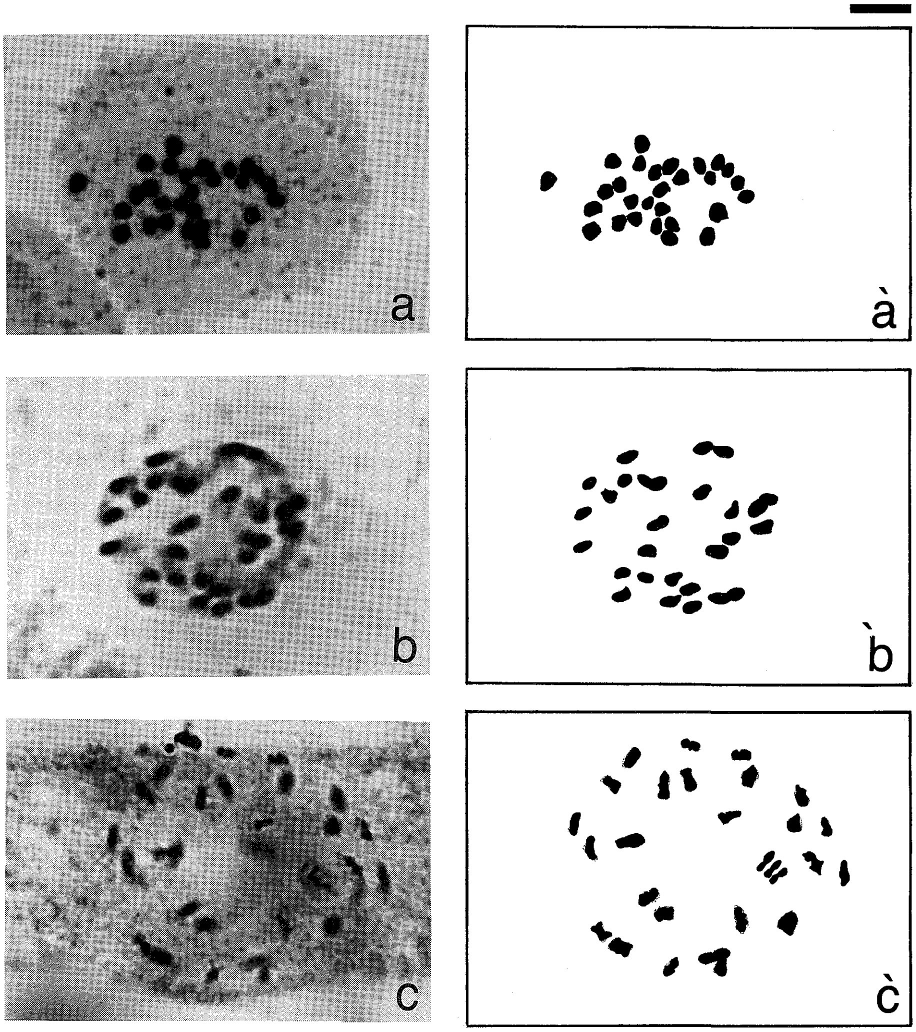

Fig. 2. Chromosomes of *Boehmeria*. a, à. *B. densiflora* var. *penduliflora* b, b̀. *B. polystachya* c, c̀. *B. ternifolia*. Scale bar = 4 μm.

1983) showed diploid individuals propagated sexually. No polyploids nor agamospermous plants are found. This unexpected result greatly differs from the ones found in Japan (Okabe 1963, Yahara 1983), where the polyploid apomicts are much more common.

In Japan and eastern China *Boehmeria* species is considered as differentiated through polyploidization accompained by apomixis and hybridization (Yahara 1990).

Present study strongly suggests that the polyploidy and apomixis has not played any role in the speciation and diversification of *Boehmeria* species in Nepal in reference to the previous studies in Japan where polyploidy and apomixis play a considerable role on the differentiation of this genus (Okabe 1963, Yahara 1983). The reason for such difference in ploidy level and mode of reproduction is at present unknown, but a

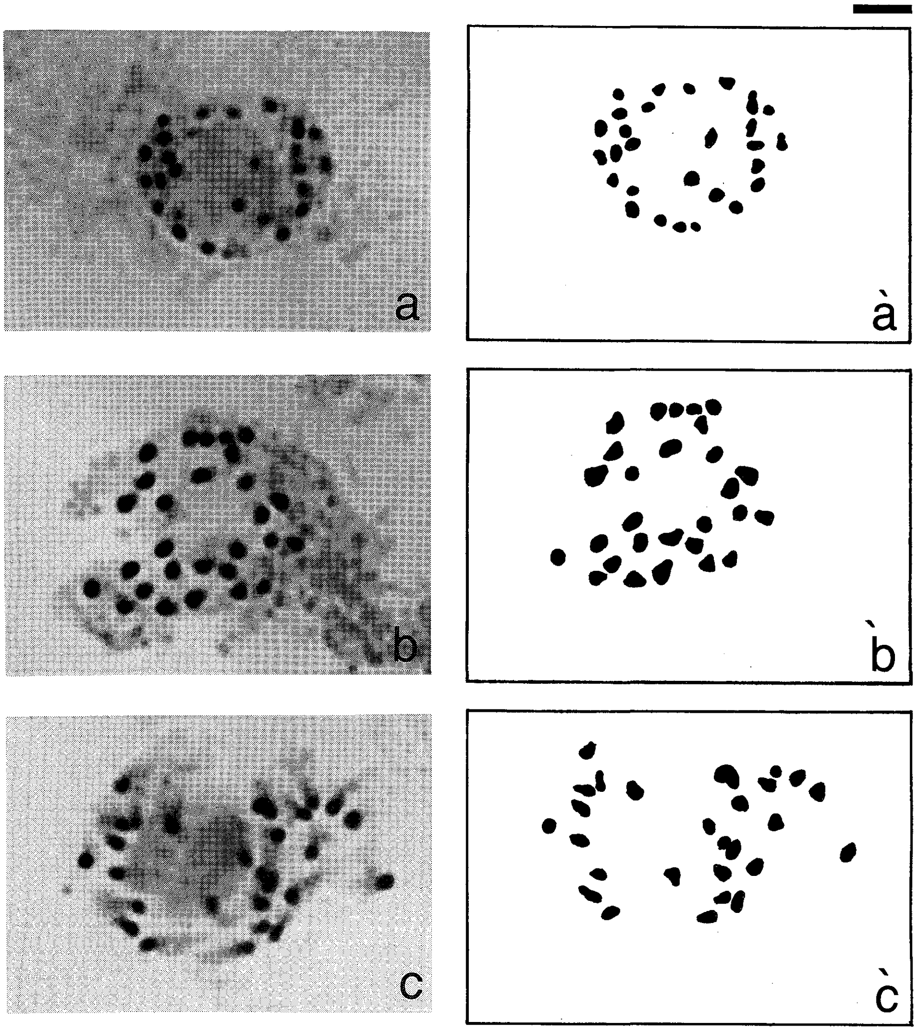

Fig. 3. Chromosomes of *Boehmeria*. a, à. *B. macrophylla* var. *macrophylla* b, b̀. *B. macrophylla* var. *rotundifolia* c, c̀. *B. macrophylla* var. *scabrella*. Scale bar = 4 μm.

few hypothesis could be proposed:

Boehmeria species growing in the Nepal Himalaya are identical with or closely related to the *Boehmeria* of South Asia (Yahara 1981). Phytogeographical studies of Chinese *Boehmeria* (Wang 1981) also pointed out that Sino-Japanese taxa has distinct distribution pattern than that of other South Asian and Himalayan *Boehmeria*. Present cytological data also reconfirms that diploid and sexual forms are common in South Asia, and Himalaya. Hence it is considered that Himalayan and Sino-Japanese *Boehmeria* belongs to two different phyletic groups. The diploids are considered as the ancestral sexual race inhabiting warm tropical regions of South Asia and the warm montane zone of Himalaya whereas polyploid agamospermous group, a derived race, distributed widely to eastern China and

Japan. *Boehmeria* species in Nepal Himalaya are distributed in the montane zone. Montane zone of Nepal are warm and humid, the climatic condition are not severe. Generally agamospermous plants are of hybrid origin and is accompanied by polyploidy (Yahara 1990). The hybridity and subsequent heterozygosity provide higher level of flexibility towards stress tolerance in agamospermous races (Asker and Jerling 1992) whereas specific habitat requirement due to the limited tolerance of sexual races limits their distribution. During the considerable environmental changes where demands on organism go beyond that which can be met with existing flexibility, continued existence could only be achieved by heterozygosity to survive the environmental challenge (Asker and Jerling 1992). Hence agamospermous race of *Boehmeria* might be widely distributed including eastern China, Japan and Korea whereas sexual race are limited to the warm humid habitat of Himalaya and South Asia.

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ナビン アチャルヤ[a]，米倉浩司[b]，鈴木三男[a,b]：

ネパール産ヤブマオ属植物の細胞分類学的研究

ネパール産ヤブマオ属植物（イラクサ科）7種16個体の根端細胞において染色体数を観察した．これらの種における染色体数の報告は初めてである．調べた全ての個体において，$2n = 28$ の染色体数が観察された．ヤブマオ属植物の染色体基本数は $x = 14$ であるので，これらの個体は二倍体であり，有性生殖を行うと考えられる．日本産ヤブマオ属に普遍的な倍数体は確認できず，このことからネパールヒマラヤにおいてはヤブマオ属植物の種分化には日本とは異なり倍数化や無融合生殖は全く関与していないものと推測される．このような違いが生じた理由は現時点では不明だが，無融合生殖型が有性生殖型よりも厳しい環境に対する適応性が高いと考えられることから，ヒマラヤの山麓の温暖で変化の少ない環境では有性生殖型が遺存しているのに対し，日本のような気候の変化の大きい環境では無融合生殖型が優占した可能性が考えられる．

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